Genomed

JetStar™ Endotoxin-free Plasmid Purification Kits

For purifying transfection-grade plasmid DNA

Cat. nos. 222010, 222020, 222100, 232006, 233006, 242006, and 243006

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User Manual

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Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Quantity	Cat. no.
JetStar™ 2.0 Endotoxin-free Maxiprep Kit	10 preps	222010
	20 preps	222020
	100 preps	222100
JetStar™ 2.0 Endotoxin-free Megaprep Kit	6 preps	232006
JetStar™ 2.0 Endotoxin-free Megaprep JetFilter Kit	6 preps	233006
JetStar™ 2.0 Endotoxin-free Gigaprep Kit	6 preps	242006
JetStar™ 2.0 Endotoxin-free Gigaprep JetFilter Kit	6 preps	243006

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

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Kit Contents and Storage, Continued

JetStar™ 2.0 Endotoxin-free Maxiprep Kits

The components included in the JetStar™ 2.0 Endotoxin-free Maxiprep Kits are listed below. **Store RNase A at 4°C and store all other components at room temperature.** See page viii for buffer compositions.

Item	Cat. no. 222010	Cat. no 222020	Cat. no. 222100
	10 preps	20 preps	100 preps
Cell Resuspending Buffer (E1)	110 mL	220 mL	2 × 550 mL
RNase A (20 mg/mL)	650 µL	1.5 mL	2 × 3 mL
Lysis Buffer (E2)	110 mL	220 mL	2 × 550 mL
Precipitation Buffer (E3)	110 mL	220 mL	2 × 550 mL
Endo-1 Buffer	33 mL	66 mL	330 mL
Equilibration Buffer (E4)	330 mL	660 mL	2 × 1,650 mL
Endo-2 Buffer	330 mL	660 mL	2 × 1,650 mL
Wash Buffer (E5)	330 mL	660 mL	2 × 1,650 mL
Elution Buffer (E6)	165 mL	330 mL	1,650 mL
Endotoxin-free Water	30 mL	30 mL	170 mL
TE Buffer	30 mL	50 mL	200 mL
Maxi Columns	10 each	20 each	100 each

Continued on next page

Kit Contents and Storage, Continued

JetStar™ 2.0 Endotoxin-free Mega- and Giga Kits

The components included in the JetStar™ 2.0 Endotoxin-free Mega- and Giga prep Kits are listed below. Note that the kits are available with and without the JetFilter. **Store RNase A at 4°C and all other components at room temperature.**

Item	Cat. no. 232006	Cat. no. 233006	Cat. no. 242006	Cat. no. 243006
	6 preps	6 preps	6 preps	6 preps
Cell Resuspending Buffer (E1)	330 mL	330 mL	825 mL	825 mL
RNase A (20 mg/mL)	3 mL	3 mL	2 × 3 mL	2 × 3 mL
Lysis Buffer (E2)	330 mL	330 mL	825 mL	825 mL
Precipitation Buffer (E3)	330 mL	330 mL	825 mL	825 mL
Endo-1 Buffer	99 mL	99 mL	250 mL	250 mL
Equilibration Buffer (E4)	660 mL	660 mL	2 × 660 mL	2 × 660 mL
Endo-2 Buffer	990 mL	990 mL	1,980 mL	1,980 mL
Wash Buffer (E5)	990 mL	990 mL	1,980 mL	1,980 mL
Elution Buffer (E6)	330 mL	330 mL	660 mL	660 mL
Endotoxin-free Water	30 mL	30 mL	30 mL	30 mL
TE Buffer	50 mL	50 mL	200 mL	200 mL
Lysate Filtration JetFilter	none	6 each	none	6 each
DNA-binding Cartridge	6 each	6 each	6 each	6 each

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Kit Contents and Storage, Continued

Buffer Composition

The composition of buffers included in the JetStar™ Endotoxin-free Kits is listed below.

Buffer	Composition
Cell Resuspending Buffer (E1)	50 mM Tris-HCl, pH 8.0 10 mM EDTA
RNase A	20 mg/mL in Buffer E1
Lysis Buffer (E2)	0.2 M NaOH 1% (w/v) SDS
Precipitation Buffer (E3)	3.1 M Potassium acetate, pH 5.5
Endo-1 Buffer	Proprietary formulation
Equilibration Buffer (E4)	0.1 M Sodium acetate, pH 5.0 0.6 M NaCl 0.15% (v/v) Triton® X-100
Endo-2 Buffer	Proprietary formulation
Wash Buffer (E5)	0.1 M Sodium acetate, pH 5.0 0.8 M NaCl
Elution Buffer (E6)	0.1 M Tris-HCl, pH 8.5 1.25 M NaCl
TE Buffer	10 mM Tris-HCl, pH 8.0–8.5 0.1 mM EDTA

Introduction

About the Kits

The JetStar™ Technology

JetStar™ Maxi, Mega, and Giga Endotoxin-free Plasmid Purification Kits contain a unique anion exchange resin packed in a gravity flow column (maxiprep) or a vacuum-driven filter cartridge (mega- and gigaprep). JetStar™ Endotoxin-free Plasmid Kits are the ideal tool to efficiently isolate large quantities of ultrapure plasmid DNA with an endotoxin content of <0.001 endotoxin units/µg (EU/µg).

Advantages

Use of the JetStar™ Endotoxin-free Plasmid Purification Kits to isolate plasmid DNA provides the following advantages:

- Rapid and convenient purification method that allows high-yield plasmid purification in 2–3 hours
- Unique anion exchange resin technology to isolate plasmid DNA negates the need for any organic solvents, cesium chloride (CsCl), or vacuum manifolds
- Endotoxin content of purified DNA <0.001 EU/µg
- Maxiprep utilizes fast gravity flow columns and mega- and gigaprep utilize ultra-quick vacuum cartridges
- No additional incubation times compared to the standard JetStar™ Plasmid Purification protocol
- Bacterial lysate remains clear during endotoxin removal
- Buffers crucial for endotoxin removal are certified to contain <0.1 EU/mL
- No chromosomal DNA contamination
- Ability to purify all types and sizes of plasmid DNA
- Reliable performance of the purified plasmid DNA in a variety of downstream applications

Continued on next page

About the Kits, Continued

Procedure Overview

The JetStar™ Maxi, Mega, and Giga Endotoxin-free Plasmid Purification Kits use a patented anion exchange resin to purify plasmid DNA. The patented resin provides excellent capacity with fast flow rates, high resolution, high yield, and efficient endotoxin removal. The isolated plasmid DNA has a purity equivalent to two passes through CsCl gradients. The general procedure is carried out as follows:

1. *E. coli* cells are harvested, resuspended in Cell Resuspending Buffer (E1) with RNase, and then lysed with Lysis Buffer (E2).
2. Precipitation Buffer (E3) is added to the lysate and the lysate is clarified by centrifugation (Maxi-, Mega-, and Gigapreps using centrifugation) or by passing over a vacuum-assisted pre-packed lysate binding filter (Mega- and Gigaprep Using JetFilters).
3. Endotoxin is preliminarily removed with Endo-1 Buffer.
4. The cleared lysate is then passed through a pre-packed anion exchange column. The negatively charged phosphates on the backbone of the DNA interact with the positive charges on the surface of the resin. The temperature, salt concentration, and pH of the solutions influence binding. Under moderate salt conditions, plasmid DNA remains bound to the resin while residual endotoxin is washed away with Endo-2 Buffer.
5. RNA, proteins, carbohydrates and other impurities are then washed away with Wash Buffer (E5).
6. The plasmid DNA is eluted under high salt conditions with the Elution Buffer (E6).
7. The eluted DNA is desalted and concentrated with an alcohol precipitation step.

The entire protocol can be completed in 2–3 hours.

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About the Kits, Continued

System Specifications

JetStar™ Endotoxin-free Plasmid Purification Kits have the following specifications:

- May be used for all sizes of plasmid DNA
- $A_{260}/A_{280} > 1.85$

Specification	Maxiprep	Megaprep	Gigaprep
Starting Culture Volume	100–200 mL	500 mL	2.5 L
Column Procedure Time	45 minutes	10 minutes	10 minutes
JetFilter Reservoir Capacity	n/a	230 mL	450 mL
JetStar™ DNA-Binding Cartridge Reservoir Capacity	60 mL	200 mL	400 mL
Elution Volume	15 mL	50 mL	100 mL
DNA Yield	up to 750 µg	up to 2.5 mg	up to 10 mg
DNA Recovery	90–95%	90–95%	90–95%

Downstream Applications

DNA purified using JetStar™ Plasmid Purification Kits is at a purity equivalent to two passes through CsCl gradients and is suitable for use in a variety of downstream applications, including those requiring the highest purity, such as:

- Transfection of primary cells, including neuronal cells
- Microinjection into animals and eukaryotic cells
- Gene-therapy
- Vaccination
- Automated and manual DNA sequencing
- Cloning
- *in vitro* transcription
- Restriction digestion

Methods

Before Starting

Introduction

Review the information in this section before starting. Guidelines are included for growing the overnight cell culture and for determining the appropriate amounts of starting material based on the plasmid copy number used.

Some buffers in the JetStar™ Endotoxin-free Plasmid Purification Kits contain hazardous chemicals.

Always wear a laboratory coat, disposable gloves, and eye protection when handling the buffers.

Important: To avoid the possibility of implosion, do not use vessels that are not designed for use with vacuum. Do not use vessels that are cracked or scratched. Always wear safety glasses when working near a bottle or flask under vacuum.

Plasmid Yields

All types and sizes of plasmid DNA can be prepared, but yields depend on the type of plasmid, copy number, the bacterial strain and the volume of bacterial culture used.

Plasmid Type and Copy Number

Use a high copy number plasmid to obtain a good yield of purified plasmid DNA. If you use a low copy number plasmid, use a higher volume of cell culture, as directed in the protocol. High copy number plasmids typically yield >3–5 µg DNA/mL LB culture grown overnight. Low copy number plasmids typically yield 0.2–1 µg DNA/mL LB culture grown overnight.

Although the JetStar™ system is compatible with other growth media, including rich media, we strongly recommend growing transformed *E. coli* cells overnight in buffered LB (Luria-Bertani) media for optimal results. Use a bacterial culture in stationary phase. The cell density should be approximately 1×10^9 cells per mL medium (1–1.5 A_{600} units/mL).

Continued on next page

Before Starting, Continued

Follow the recommendations below to obtain the best results:

- Ensure that no DNase is introduced into the sterile buffers supplied with the kit.
- Sterilize all equipment coming in contact with DNA, including pipette tips and tubes and maintain a sterile environment when handling DNA.
- Perform the recommended wash steps during purification to obtain the best results.
- To troubleshoot, save aliquots as recommended during the purification procedure, see page 31.

Creating an endotoxin-free environment

- Bake all glassware for at least 6 hours at ≥200°C in an oven. Autoclaving does NOT destroy endotoxins. Since autoclaves are normally used to decontaminate bacterial cultures, they are a common source of endotoxins.
- Use only plasticware that is certified to be endotoxin- or pyrogen-free by the respective vendor.
- If preparing buffers and solutions yourself, all chemicals should be taken from fresh unopened bottles. Reagents used to prepare endotoxin-free buffers should be marked accordingly to keep the chemicals endotoxin-free.

Note

When working with gram-negative bacteria in the lab, endotoxins are likely to be present on nearly all parts that are in use by multiple researchers (e.g., many parts of glassware). Endotoxin molecules are very resistant against physical means of inactivation (e.g., heat). Therefore, consider even single-use items (e.g., plasticware disposables) to be contaminated with endotoxins if not expressively otherwise stated.

Following elution, be very careful not to re-contaminate plasmid DNA again by using endotoxin-contaminated water, chemicals, glass or plasticware.

Endotoxin-free Maxiprep

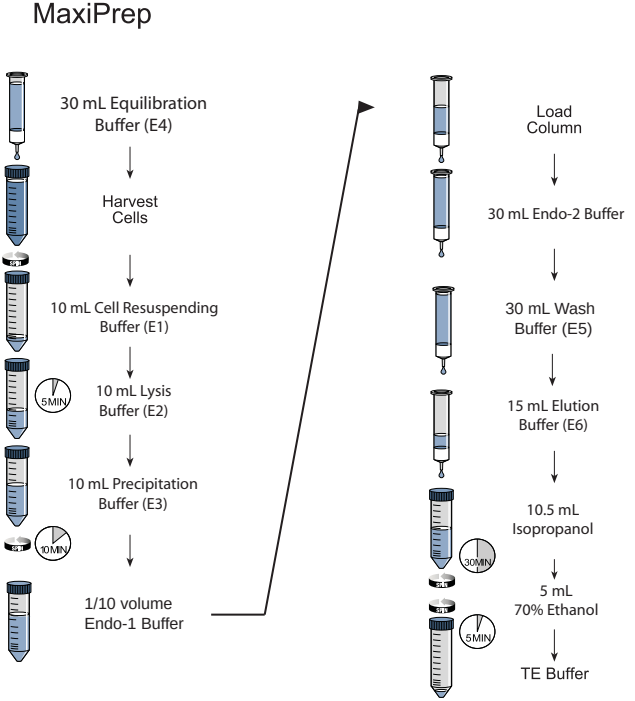
Experimental Overview

Introduction

Up to 750 µg of high-quality plasmid DNA may be purified with the JetStar™ Endotoxin-free Plasmid Maxiprep Kit in ~2 hours from overnight *E. coli* cultures.

JetStar™ 2.0 Endotoxin-free Maxiprep Kit

The flow chart for purifying plasmid DNA using the JetStar™ 2.0 Endotoxin-free Maxiprep Kit is shown below.



Maxiprep Procedure

Components Supplied with the Kit

- Cell Resuspending Buffer (E1) with RNase A (see page 8)
- Lysis Buffer (E2)
- Precipitation Buffer (E3)
- Endo-1 Buffer
- Equilibration Buffer (E4)
- Endo-2 Buffer
- Wash Buffer (E5)
- Elution Buffer (E6)
- TE Buffer
- Endotoxin-free water for preparing 70% ethanol
- JetStar™ Maxi Columns

Materials Supplied by the User

- Overnight culture of transformed *E. coli* cells
- Isopropanol
- Sterile, endotoxin- or pyrogen-free microcentrifuge tubes
- JetRack (page 34).
- Tubes or buckets of appropriate size for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Appropriate 50 mL centrifuge tube capable of withstanding centrifugation forces > 12,000 × *g*
- Centrifuge capable of centrifuging at >12,000 × *g* at 4°C
- *Optional:* PureLink™ HiPure Precipitator Module (page 34)

Continued on next page

Maxiprep Procedure, Continued

Purification Rack

The JetRack is designed for use with the JetStar™ Plasmid DNA Maxiprep Kits (see page 34 for ordering information). The JetRack consists of a Column Holder Rack (for processing 4 maxiprep columns simultaneously), a Collection Tube Rack, and a Waste Container for collecting waste.

Overnight Culture

Inoculate a plasmid-containing *E. coli* culture according to good microbiological practice in growth medium with the appropriate antibiotic and grow the cells for 16–18 hours (usually overnight) or 24–36 hours if using a buffered growth medium.

- For **high copy number plasmids**, use 100 mL of an overnight LB culture per sample.
- For **low copy number plasmids**, use 250–500 mL of an overnight LB culture per sample.

  Note: For culture volumes >200 mL, add twice the amount of Cell Resuspending Buffer (E1) with RNase A, Lysis Buffer (E2), and Precipitation Buffer (E3) as directed in Steps 2, 3, and 4, next page.

Preparing Buffers and Solutions

Cell Resuspending Buffer (E1)

Add RNase A to the Cell Resuspending Buffer (E1) according to the note with the RNase A tube. Mix well. The final RNase A concentration is 100 µg/mL. Mark the bottle label to indicate that RNase A is added and store at 4°C.

Lysis Buffer (E2)

Check the Lysis Buffer (E2) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

70% Ethanol

Prepare 70% ethanol with the endotoxin-free water supplied with the kit. Add absolute (96–100%) ethanol as stated on the label of the bottle and mix thoroughly.

Continued on next page

Maxiprep Procedure, Continued

Equilibrating the Column

Place the JetStar™ Maxi column on the JetRack (see the manual supplied with the rack for more details). Apply 30 mL Equilibration Buffer (E4) to the column. Allow the solution in the column to drain by gravity flow. Equilibration takes about 10–15 minutes, but does not influence the speed of the protocol; therefore, proceed to Step 1 (below) during equilibration.

Preparing Cell Lysate

1. Harvest the cells by centrifuging the overnight LB-culture at 12,000 × *g* for 3 minutes in a bucket. Remove all medium.
2. Add 10 mL Cell Resuspending Buffer (E1) with RNase A to the pellet and resuspend the cells until homogeneous. Transfer cell suspension to a 50-mL centrifuge tube.

   Note: If overnight culture volume >200 mL, use 20 mL Cell Resuspending Buffer (E1).
3. Add 10 mL Lysis Buffer (E2). Mix gently by inverting the capped tube until the mixture is homogenous. **Do not vortex.** Incubate at room temperature for 5 minutes.

   Note: Do not allow lysis to proceed for more than 5 minutes. If overnight culture volume >200 mL, use 20 mL Lysis Buffer (E2).
4. Add 10 mL Precipitation Buffer (E3) and mix immediately by inverting the capped tube until the mixture is homogeneous. The viscous matter present after cell lysis (step 3) should not remain. **Do not vortex.**

   Note: When many samples are prepared in parallel, mix each sample immediately after adding Precipitation Buffer (E3). If overnight culture volume >200 mL, use 20 mL Precipitation Buffer (E3).

Continued on next page

Maxiprep Procedure, Continued

Preparing Cell Lysate, Continued

5. Centrifuge the mixture at >12,000 × *g* for 10 minutes at room temperature.

   Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to separate the lysate and pellet. Pipette the clear lysate into another sterile tube and centrifuge at >12,000 × *g* for 5 minutes at room temperature to remove any remaining cellular debris.

6. Proceed to **Removing Endotoxin**, below.

Removing Endotoxin

1. Mix the clear supernatant with 1/10 volume of Endo-1 Buffer.

   For example, add 3 mL of Endo-1 Buffer to 30 mL cleared lysate. Mix well by inverting.

   Note: Mix the cleared lysate and Endo-1 Buffer to homogeneity.

2. Load the supernatant onto the equilibrated column with a pipette. Allow the solution in the column to drain by gravity flow.
3. Wash the column with 30 mL Endo-2 Buffer. Allow the solution in the column to drain by gravity flow. Discard the flow-through.
4. Wash the column with 30 mL Wash Buffer (E5) as described above.
5. Proceed to **Eluting and Precipitating DNA** (next page).

Note

The PureLink™ HiPure Precipitator Module (page 34) may be used for precipitation in 10 minutes without any centrifugation steps. Alternatively, follow the protocol on the next page to perform traditional DNA precipitation using centrifugation.

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Maxiprep Procedure, Continued

Eluting and Precipitating DNA

1. Place a sterile, endotoxin- or pyrogen-free 30-mL centrifuge tube (elution tube) under the column.
2. Add 15 mL Elution Buffer (E6) to the column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. *The elution tube contains the purified DNA*. Discard the column.
3. Add 10.5 mL isopropanol to the elution tube. Mix well.

   Note: Proceed to the protocol described in the PureLink™ HiPure Precipitator manual after this step if you are using the precipitator.
4. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Wash the DNA pellet in 5 mL endotoxin-free 70% ethanol.
6. Centrifuge at >12,000 × *g* for 5–10 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10–15 minutes at room temperature or 65–70°C.

   Note: Be careful when drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber. The DNA will not redissolve completely if it is overdried.
8. Resuspend the DNA pellet in a suitable volume of endotoxin-free TE Buffer (supplied with the kit) or endotoxin-free 10 mM Tris-HCl, or endotoxin-free water.

Storing DNA

To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use. For long-term storage, aliquot the DNA and store at –20°C.

Endotoxin-free Megaprep

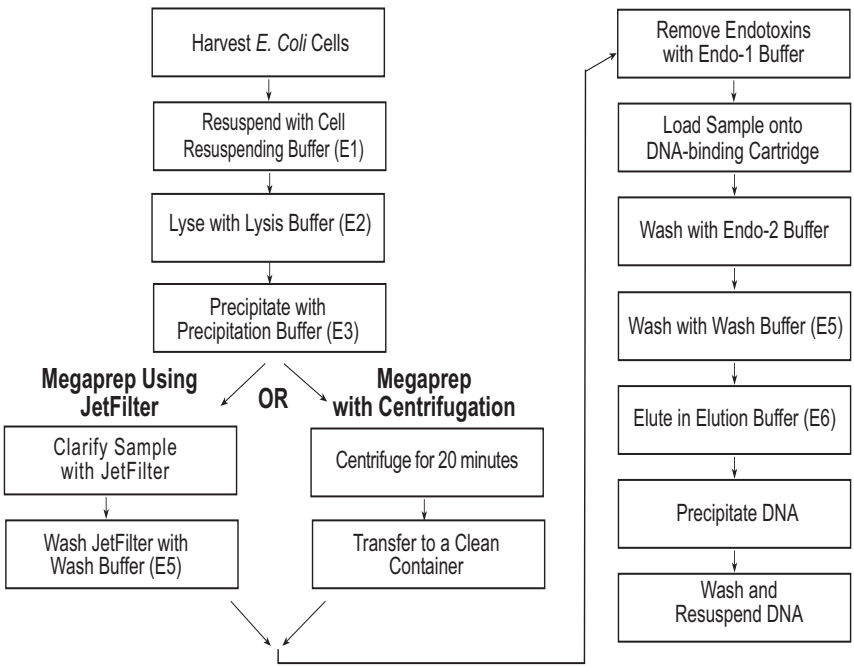
Experimental Overview

Introduction

Up to 2.5 mg of high-quality plasmid DNA may be purified with the JetStar™ Endotoxin-free Plasmid Megaprep Kit. The JetStar™ Megaprep procedure is completed in 2–3 hours using 500 mL of high copy number starting bacterial culture, or 2.5 L of low copy number starting bacterial culture.

Flow Chart

The flow chart for purifying plasmid DNA using the JetStar™ 2.0 Endotoxin-free Megaprep Kits is shown below.


Harvest E. Coli Cells
Resuspend with Cell Resuspending Buffer (E1)
Lyse with Lysis Buffer (E2)
Precipitate with Precipitation Buffer (E3)
Megaprep Using JetFilter
OR
Megaprep with Centrifugation
Clarify Sample with JetFilter
Centrifuge for 20 minutes
Wash JetFilter with Wash Buffer (E5)
Transfer to a Clean Container
Remove Endotoxins with Endo-1 Buffer
Load Sample onto DNA-binding Cartridge
Wash with Endo-2 Buffer
Wash with Wash Buffer (E5)
Elute in Elution Buffer (E6)
Precipitate DNA
Wash and Resuspend DNA

Megaprep Procedure

Components Supplied with the Kit

- Cell Resuspending Buffer (E1) with RNase A (page 15)
- Lysis Buffer (E2)
- Precipitation Buffer (E3)
- Endo-1 Buffer
- Equilibration Buffer (E4)
- Endo-2 Buffer
- Wash Buffer (E5)
- Elution Buffer (E6)
- TE Buffer
- Endotoxin-free water for preparing 70% ethanol
- Megaprep JetFilter (Cat. no. 233006 only)
- JetStar™ DNA-Binding Cartridge

Note

- The JetFilter for clarification of the bacterial lysate contains a yellow ring.
- JetStar™ DNA-Binding Cartridges with endotoxin-free ion-exchange chromatography material for purifying DNA contain a red ring.

Materials Supplied by the User

- Overnight culture of transformed *E. coli* cells
- Isopropanol
- Sterile, endotoxin- or pyrogen-free microcentrifuge tubes
- Tubes or buckets of appropriate size for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Sterile 50-mL centrifuge tubes
- Sterile spatula
- Sterile container for transferring the lysate

Continued on next page

Megaprep Procedure, Continued

Materials Supplied by the User, Continued

- Vacuum source capable of generating a negative pressure of –600 to –800 mbar and equipped with a vacuum regulator.

  Note: If the vacuum pump is not equipped with a vacuum regulator, you may need to purchase a regulator, such as the PureLink™ vacuum regulator (see page 34), and attach the regulator to the vacuum pump.
- 1 L flask or a 1,000-mL Stericup Receiver flask with 45-mm thread (Millipore Cat. no. SC00B10RE)
- Sterile, endotoxin-free 250 mL flask with 45 mm thread
- For plasmid purification using the JetFilter (Cat. no. 233006), you will also need a 500 mL flask or a 500-mL Stericup Receiver Flask with 45-mm thread (Millipore Cat. no. SC00B05RE)
- *Optional:* PureLink™ HiPure Precipitator Module (page 34)

Overnight Culture

Inoculate a plasmid-containing *E. coli* culture according to good microbiological practice in growth medium with the appropriate antibiotic and grow the cells for 16–18 hours or for 24–36 hours if using a buffered growth medium.

- For **high copy number plasmids**, use 500 mL of an overnight LB culture per sample.
- For **low copy number plasmids**, use 2,500 mL of an overnight LB culture per sample.

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Megaprep Procedure, Continued

Preparing Buffers and Solutions

Cell Resuspending Buffer (E1)

Add RNase A to the Cell Resuspending Buffer (E1) according to the note with the RNase A tube. Mix well. The final RNase A concentration is 100 µg/mL. Mark the bottle label to indicate that RNase A is added. Store Cell Resuspending Buffer (E1) with RNase A at 4°C.

Lysis Buffer (E2)

Check the Lysis Buffer (E2) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

70% Ethanol

Prepare 70% ethanol with the endotoxin-free water supplied with the kit. Add the appropriate amount of absolute (96–100%) ethanol as stated on the label of the bottle and mix thoroughly.

Preparing the JetFilter

1. Attach a JetFilter (with a yellow ring) onto a clean 500 mL bottle with a 45 mm neck. Do not overtighten the cartridge on the bottle neck since the cartridge plastic may crack.
2. Set cartridge aside for later use (**Clarifying the Lysate Using the JetFilter,** page 17).

Preparing the JetStar™ DNA-Binding Cartridge

1. Attach a JetStar™ Megaprep DNA-Binding Cartridge (with a red ring) onto a clean 1,000 mL Stericup receiver flask or a 1 Liter bottle with 45 mm neck. Do not overtighten the cartridge on the bottle neck, since the cartridge plastic may crack.
2. Connect the vacuum source to the tubing connector of filtration cartridge. An example of the set up with a JetStar™ Megaprep DNA-Binding Cartridge is shown in the figure on the next page.

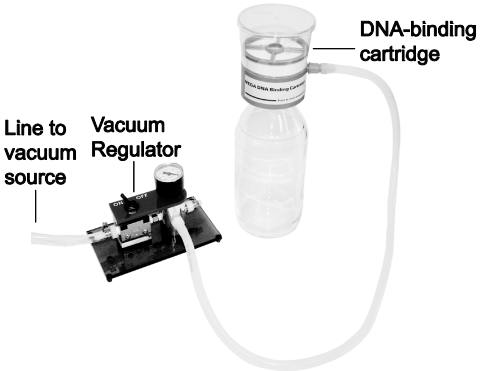
   Note: Make sure the vacuum source has a vacuum regulator to adjust the vacuum pressure during the procedure (see page 34).

Continued on next page

Megaprep Procedure, Continued

Preparing the JetStar™ DNA-Binding Cartridge, Continued

3. Equilibrate the JetStar™ DNA-Binding Cartridge with 100 mL Equilibration Buffer (E4).
4. Apply vacuum to the cartridge through the side-arm with tubing-connector. Keep vacuum on until all liquid has drained from the resin. Discard the flow-through. Set equilibrated cartridge aside for later use (**Removing Endotoxin,** page 18)



Preparing Cell Lysate

1. Harvest up to 500 mL (high copy number plasmid) or up to 2.5 L (low copy number plasmid) of *E. coli* cells by centrifuging the overnight LB-culture at 12,000 × *g* for 5 minutes in a bucket. Remove all medium.
2. Add 50 mL Cell Resuspending Buffer (E1) with RNase A to the pellet and resuspend the cells until homogeneous.

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Preparing Cell Lysate, Continued

3. Add 50 mL Lysis Buffer (E2). Mix gently by inverting the capped tube until the mixture is homogenous. **Do not vortex.** Incubate at room temperature for 5 minutes.

   Note: Do not allow lysis to proceed for more than 5 minutes.

4. Add 50 mL Precipitation Buffer (E3) and mix immediately by inverting the capped tube until the mixture is homogeneous. The viscous matter present after cell lysis (Step 3) should not remain. **Do not vortex**.

   Note: When many samples are prepared in parallel, each sample should be mixed immediately after adding Lysis Buffer (E3).

5. The cell lysate may be clarified using centrifugation or vacuum.

   - For Cat. no. 232006, clarify the lysate by centrifuging the lysate at >12,000 × *g* for 20 minutes at room temperature and transfer the particle-free supernatant into a clean container. Proceed directly to **Removing Endotoxin,** page 18.
   - For Cat. no. 233006, clarify the lysate using the JetFilter as directed in **Clarifying the Lysate Using the JetFilter**, below.

Clarifying the Lysate Using the JetFilter

1. Pour the neutralized bacterial lysate directly into the prepared JetFilter with the yellow ring (see **Preparing the JetFilter,** page 15).
2. Let stand at room temperature for at least 10 minutes without agitation.

   Note: Let the lysate stand for at least 10 minutes after the transfer into the cartridge to allow the precipitate to float and form a layer on top of the lysate. If necessary, extend the incubation time of the lysate in the cartridge until the layer has formed to prevent clogging and ensure efficient filtration.

Continued on next page

Clarifying the Lysate Using the JetFilter, Continued

3. Connect the vacuum source to the tubing connector of the filtration cartridge and apply vacuum (−600 to −800 mbar). Collect the flow-through into the flask. Keep the vacuum on until all liquid has drained. Turn off vacuum.

   Note: An unclogged JetFilter filters 125 mL of filtrate in 1–2 minutes when vacuum is applied.
4. Add 50 mL of Wash Buffer (E5) to the JetFilter and gently stir the precipitate with a sterile spatula to improve the flow of liquid through the filter unit.
5. Apply vacuum until all liquid has drained from the filtration cartridge.

   The receiving flask contains the clarified lysate with the plasmid DNA.
6. Remove and discard the JetFilter into biohazardous waste. Pour the contents of the receiving flask (filtered lysate) into a new sterile container.
7. Proceed to **Removing Endotoxin**, below.

Removing Endotoxin

1. Mix the supernatant with 1/10 volume of Endo-1 Buffer.

   For example, add 15 mL of Endo-1 Buffer to 150 mL cleared lysate. Mix well by inverting until the mixture is homogenous.
2. Load the supernatant onto the equilibrated JetStar™ DNA-Binding Cartridge with a red ring (from Step 4, page 16).
3. Apply vacuum to the cartridge. Keep the vacuum on until all liquid has drained from the resin. Take an aliquot (100 µL) of the flow-through for further analysis.
4. Add 150 mL of Endo-2 Buffer to the cartridge and apply vacuum. Keep the vacuum on until all liquid has drained from the resin. Take a 100 µL aliquot of the flow-through.

Continued on next page

Megaprep Procedure, Continued

Removing Endotoxin, Continued

5. Repeat step 4 using 150 mL Wash Buffer (E5). Discard the flow-through. *The binding cartridge contains the DNA*.
6. Proceed to **Eluting and Precipitating DNA** (below).

Note

The PureLink™ HiPure Precipitator Module (page 34) may be used for precipitation in 10 minutes without any centrifugation steps. Alternatively, follow the protocol on the nest page to perform traditional DNA precipitation using centrifugation.

Eluting and Precipitating DNA

1. Remove the JetStar™ DNA-Binding Cartridge from the waste bottle and attach it onto a clean, sterile, endotoxin-free 250-mL Stericup Receiver Flask.
2. Add 50 mL Elution Buffer (E6) into the cartridge.
3. Apply soft vacuum (–100 to –200 mbar) to the cartridge through the side-arm until approximately 5–10 mL of Elution Buffer (E6) has passed through the cartridge. Then release the vacuum from the cartridge, so that no further liquid is pulled through the resin.
4. Let stand for 1 minute without agitation.
5. Apply vacuum again and draw the remaining liquid from the resin into the receiver bottle. Keep vacuum on for approximately 5 minutes until all liquid has drained from the resin.
6. *Optional Elution*: Add an additional 50 mL Elution Buffer (E6) to the JetStar™ DNA-Binding Cartridge. Repeat Steps 3–5.

   Note: This second elution step can increase the final DNA yield by approximately 10–15%.

   The 250-mL receiver flask now contains the eluate with the purified DNA. Save a 100 µL aliquot of the eluate for further analysis.

Continued on next page

Eluting and Precipitating DNA, Continued

7. Transfer the DNA eluate into several 50-mL centrifuge tubes (maximum 17.5 mL per tube).
8. Add 0.7 volumes of isopropanol and mix well.

   Note: Proceed to the protocol described in the PureLink™ HiPure Precipitator manual after this step if you are using the precipitator.
9. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Carefully remove and discard the supernatant.

   Note: Perform centrifugations with a swinging bucket rotor that allows >12,000 × *g* to prevent the plasmid DNA from sticking to the walls of the centrifuge tube. If such a rotor is not available, siliconize the centrifuge tubes with a repellent silane (dimethyldichlorosilane).
10. Wash the DNA pellet in 10–15 mL endotoxin-free 70% ethanol. Vortex gently.
11. Centrifuge at >12,000 × *g* for 10 minutes at 4°C. Carefully remove and discard the supernatant.
12. Air-dry the pellet for 10–15 minutes at room temperature or 65–70°C.

    Note: Be careful when drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber. The DNA will not be redissolved completely if it is overdried.
13. Resuspend the DNA pellet in a suitable volume of endotoxin-free TE Buffer (supplied with the kit) or endotoxin-free 10mM Tris-HCl or endotoxin-free water.

Storing DNA

To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use. For long-term storage, aliquot the DNA and store at –20°C.

Endotoxin-free Gigaprep

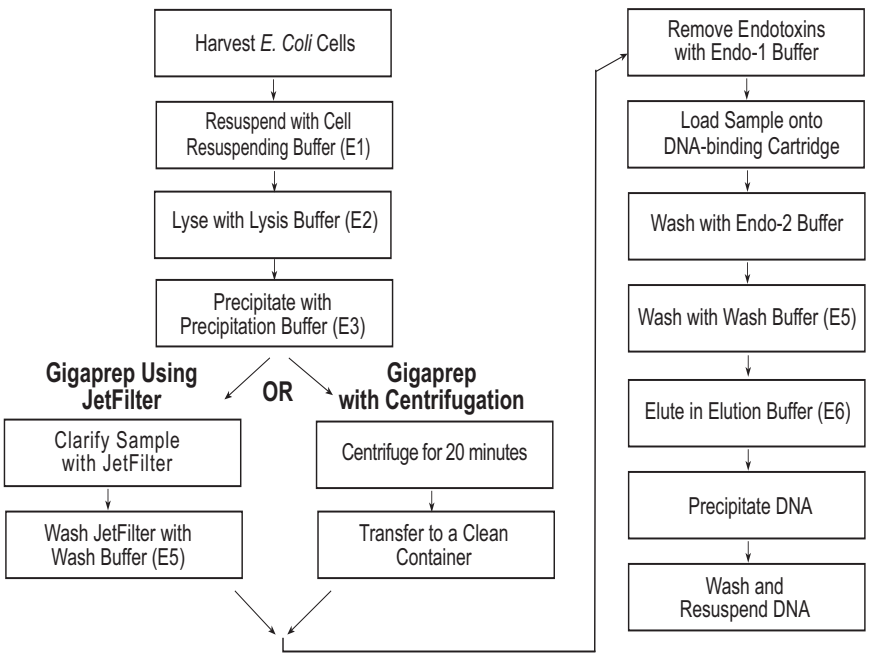
Experimental Overview

Introduction

Up to 10 mg of high-quality plasmid DNA may be purified with the JetStar™ Endotoxin-free Plasmid Gigaprep Kit in 2–3 hours using 2.5 L of high copy number starting bacterial culture or 5 L of low copy number starting bacterial culture.

Flow Chart

The flow chart for purifying plasmid DNA using the JetStar™ 2.0 Endotoxin-free Gigaprep Kit is shown below.


Harvest *E. Coli* Cells
Resuspend with Cell Resuspending Buffer (E1)
Lyse with Lysis Buffer (E2)
Precipitate with Precipitation Buffer (E3)
Gigaprep Using JetFilter
OR
Gigaprep with Centrifugation
Clarify Sample with JetFilter
Centrifuge for 20 minutes
Wash JetFilter with Wash Buffer (E5)
Transfer to a Clean Container
Remove Endotoxins with Endo-1 Buffer
Load Sample onto DNA-binding Cartridge
Wash with Endo-2 Buffer
Wash with Wash Buffer (E5)
Elute in Elution Buffer (E6)
Precipitate DNA
Wash and Resuspend DNA

Gigaprep Procedure

Components Supplied with the Kit

- Cell Resuspending Buffer (E1) with RNase A (page 23)
- Lysis Buffer (E2)
- Precipitation Buffer (E3)
- Endo-1 Buffer
- Equilibration Buffer (E4)
- Endo-2 Buffer
- Wash Buffer (E5)
- Elution Buffer (E6)
- TE Buffer
- Endotoxin-free water for preparing 70% ethanol
- Gigaprep JetFilter (for Cat. no. 243006 only)
- JetStar™ Gigaprep DNA-Binding Cartridge

Note

- JetFilters for clarification of the bacterial lysate contain a yellow ring.
- JetStar™ Gigaprep DNA-Binding Cartridges with endotoxin-free ion-exchange chromatography material for purifying DNA contain a red ring.

Materials Supplied by the User

- Overnight culture of transformed *E. coli* cells
- Isopropanol
- Sterile, endotoxin- or pyrogen-free microcentrifuge tubes
- Tubes or buckets of appropriate size for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- Sterile 50-ml centrifuge tubes
- Sterile spatula
- Sterile container for transferring the lysate

Continued on next page

Gigaprep Procedure, Continued

Materials Supplied by the User, Continued

- Vacuum source capable of generating a negative pressure of –600 to –800 mbar and equipped with a vacuum regulator.

  Note: If the vacuum pump is not equipped with a vacuum regulator, you may need to purchase a regulator, such as the PureLink™ vacuum regulator (see page 34) and attach the regulator to the vacuum pump.
- 1 L flask or a 1,000-mL Stericup receiver flask with 45-mm thread (Millipore Cat. no. SC00B10RE)

  Note: You will need two 1 Liter flasks for each sample if you are using a JetFilter for lysate clarification
- Sterile, endotoxin-free 250 mL flask with 45 mm thread
- *Optional:* PureLink™ HiPure Precipitator Module (page 34)

Preparing Buffers and Solutions

Cell Resuspending Buffer (E1)

Add RNase A to the Cell Resuspending Buffer (E1) according to the note with the RNase A tube. Mix well. The final RNase A concentration is 100 µg/mL. Mark the bottle label to indicate that RNase A is added. Store Cell Resuspending Buffer (E1) with RNase A at 4°C.

Lysis Buffer (E2)

Check the Lysis Buffer (E2) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

70% Ethanol

Prepare 70% ethanol with the endotoxin-free water supplied with the kit. Add the appropriate amount of absolute (96–100%) ethanol as stated on the label of the bottle and mix thoroughly.

Continued on next page

Gigaprep Procedure, Continued

Overnight Culture

Inoculate an *E. coli* culture according to good microbiological practice in growth medium with the appropriate antibiotic and grow the cells for 16–18 hours (usually overnight) or 24–36 hours if using a buffered growth medium.

- For **high copy number plasmids**, use 2,500 mL of an overnight LB culture per sample.
- For **low copy number plasmids**, use 5,000 mL of an overnight LB culture per sample.

Preparing the DNA-binding Cartridge

1. Attach a JetStar™ DNA-Binding Cartridge (with a red ring) onto a clean 1,000 mL Stericup receiver flask or a 1 Liter bottle with 45 mm neck. Do not overtighten the cartridge on the bottle neck, since the cartridge plastic may crack.
2. Connect the vacuum source to the tubing connector of the filtration cartridge. An example of the set up with a JetStar™ DNA-Binding Cartridge is shown in the figure on the next page.

   Note: Make sure the vacuum source has a vacuum regulator to adjust the vacuum pressure during the procedure (see page 34).
3. Equilibrate the JetStar™ DNA-Binding Cartridge with 200 mL Equilibration Buffer (E4).
4. Apply vacuum to the cartridge through the side-arm with tubing-connector. Keep vacuum on until all liquid has drained from the resin. Discard the flow-through. Set equilibrated cartridge aside for later use (**Removing Endotoxin,** page 27).

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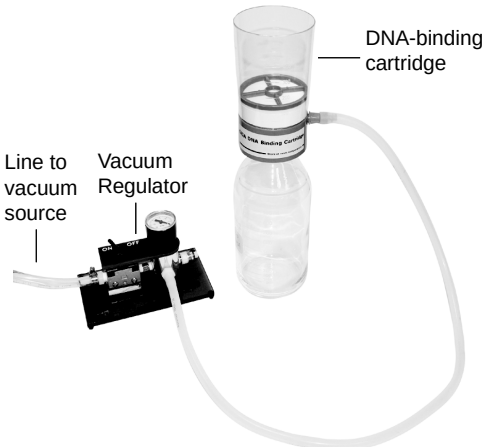
Gigaprep Procedure, Continued

JetStar™ DNA-Binding Cartridge



Preparing the JetFilter

1. Attach a JetFilter (with a yellow ring) onto a clean 1,000 mL bottle with a 45 mm neck. Do not overtighten the cartridge on the bottle neck since the cartridge plastic may crack.
2. Set cartridge aside for later use (see Step 5, page 26).

Preparing Cell Lysate

1. Harvest up to 2.5 L (high copy number plasmids) or up to 5 L (low copy number plasmids) of *E. coli* cells from overnight LB cultures by centrifuging cells in a bucket at 12,000 x *g* for 5 minutes at 4°C. Remove all medium.
2. Add 125 mL Cell Resuspending Buffer (E1) with RNase A to the pellet and resuspend the cells until homogeneous.
3. Add 125 mL Lysis Buffer (E2). Mix gently by inverting the capped tube until the mixture is homogenous. **Do not vortex.** Incubate at room temperature for 5 minutes.

   Note: Do not allow lysis to proceed for more than 5 minutes.

Continued on next page

Preparing Cell Lysate, Continued

4. Add 125 mL Precipitation Buffer (E3) and mix immediately by inverting the capped tube until the mixture is homogeneous. The viscous matter present after cell lysis (Step 3) should not remain. **Do not vortex**. A white, flocculent precipitate of proteins, cellular debris, genomic DNA, and detergent is formed.

   Note: When many samples are prepared in parallel, mix each sample immediately after adding Precipitation Buffer (E3).

5. Clarify the cell lysate using centrifugation or vacuum.

   - For Cat. no. 242006, clarify the lysate by centrifuging at >12,000 × *g* for 20 minutes at room temperature and transfer the particle-free supernatant into a clean container. Proceed directly to **Removing Endotoxin**, page 27.
   - For Cat. no. 243006, clarify the lysate with a JetFilter as described in Steps a–g, below.

   a. Pour the neutralized bacterial lysate directly into the JetFilter with the yellow ring (see **Preparing the JetFilter,** page 25).

   b. Incubate at room temperature for at least 10 minutes without agitation.

      Note: Let the lysate stand for at least 10 minutes after the transfer into the JetFilter to allow the precipitate to float and form a layer on top of the lysate. If necessary, extend the incubation time of the lysate in the JetFilter until the layer has formed to prevent clogging and ensure efficient filtration.

   c. Connect the vacuum source to the tubing connector of the JetFilter and apply vacuum (–600 to –800 mbar) until all liquid has drained. Turn off the vacuum.

      Note: An unclogged JetFilter filters 125 mL of filtrate in 1–2 minutes when vacuum is applied.

Continued on next page

Gigaprep Procedure, Continued

Preparing Cell Lysate, Continued

d. Add 50 mL of Wash Buffer (E5) to the JetFilter and gently stir the precipitate with a sterile spatula to improve the flow of liquid through the filter unit.

e. Connect the vacuum source again and apply vacuum until all liquid has been pulled through completely. Save a 100 µL aliquot of the flow-through for further analysis.

f. Close the bottle and mix the contents well by shaking. (Gentle agitation of the precipitate improves the flow of liquid through the filter unit). The bottle now contains the clear filtered bacterial lysate with the plasmid DNA. When filtration is finished, discard the single-use JetFilter.

g. Proceed to **Removing Endotoxin**, below.

Removing Endotoxin

1. To the supernatant, add 1/10 volume of Endo-1 Buffer and mix well to homogeneity. For example, add 35 mL of Endo-1 Buffer to 350 mL cleared lysate. Mix well by inverting.
2. Load the supernatant onto the equilibrated JetStar™ DNA-Binding Cartridge with a red ring (from Step 4, page 24). Apply vacuum to the cartridge through the side-arm with tubing-connector. Keep the vacuum on until all liquid has drained from the resin. Take an aliquot (100 µL) of the flow-through for further analysis, if desired.
3. Add 275 mL of Endo-2 Buffer to the JetStar™ DNA-Binding Cartridge and apply vacuum. Keep the vacuum on until all liquid has drained from the resin. Take a 100 µL aliquot of the flow-through for further analysis, if desired.
4. Repeat Step 3 using 275 mL Wash Buffer (E5). Discard the flow-through. *The binding cartridge contains the DNA*.
5. Proceed to **Eluting and Precipitating DNA** (next page).

Continued on next page

Gigaprep Procedure, Continued

Note

The PureLink™ HiPure Precipitator Module (page 34) may be used for precipitation in 10 minutes without any centrifugation steps. Alternatively, follow the protocol below to perform traditional DNA precipitation using centrifugation.

Eluting and Precipitating DNA

1. Remove the JetStar™ DNA-Binding Cartridge from the waste bottle and attach it onto a clean, sterile, endotoxin-free 250-mL Stericup receiver flask (or equivalent). Add 100 mL Elution Buffer (E6) into the cartridge.
2. Apply a soft vacuum (–100 to –200 mbar) to the cartridge until approximately 20–30 mL of Elution Buffer (E6) has eluted from the cartridge. Then release the vacuum from the cartridge, so that no further liquid is pulled through the resin.
3. Let stand for 1 minute without agitation.
4. Apply vacuum again and draw the remaining liquid from the resin into the receiver bottle. Keep vacuum on for approximately 5 minutes until all liquid has drained from the resin.
5. *Optional Elution*: Add another 100 mL Elution Buffer (E6) to the cartridge and repeat steps 2–4.

   Note: This second elution step can increase the final DNA yield by approximately 10%.

   The 250-ml flask contains eluate with the purified DNA. Save a 100 µL aliquot of the eluate for further analysis.
6. Transfer the DNA eluate into several 50-mL centrifuge tubes (maximum 17.5 mL per tube).
7. Add 0.7 volumes of isopropanol and mix well.

   Note: Proceed to the protocol described in the PureLink™ HiPure Precipitator manual after this step if you are using the precipitator.

Continued on next page

Gigaprep Procedure, Continued

Eluting and Precipitating DNA, Continued

8. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Carefully remove and discard the supernatant.

   Note: Perform centrifugations with a swinging bucket rotor that allows >12,000 x *g* to prevent the plasmid DNA from sticking to the walls of the centrifuge tube. If such a rotor is not available, siliconize the centrifuge tubes with a repellent silane (dimethyldichlorosilane).
9. Wash the DNA pellet in 10–15 mL endotoxin-free 70% ethanol. Vortex gently.
10. Centrifuge at >12,000 × *g* for 10 minutes at 4°C. Carefully remove and discard the supernatant.
11. Air-dry the pellet for 10–15 minutes at room temperature.

    Note: Be careful when drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber. The DNA will not redissolve completely if it is overdried.
12. Resuspend the DNA pellet in a suitable volume of endotoxin-free TE Buffer (supplied with the kit) or endotoxin-free 10 mM Tris-HCl or endotoxin-free water.

Storing DNA

To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

Appendix

Estimating DNA Yield

Introduction

Once you have isolated DNA, you may determine the quantity of the purified DNA as described below.

DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ DNA Assay Kits (see page 34 for ordering information).

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance of the dilution at 260 nm (A_{260}) in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5.
2. Calculate the concentration of DNA using the formula:

   DNA (µg/mL) = $A_{260} \times 50 \times$ dilution factor

For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (page 34) provide a rapid, sensitive, and specific method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometer or Qubit® Fluorometer.

Troubleshooting

If problems arise during the procedure, analyze the aliquots harvested during the procedure on a 1% agarose gel to determine the presence of DNA in the aliquots. The table below lists the results and probable cause.

Aliquot	Normal Results	Abnormal Results	Cause of Abnormal Results
JetStar™ DNA-Binding Cartridge flow-through	No DNA	DNA	• Lysate at improper pH or salt concentration to bind column. • JetStar™ DNA-Binding Cartridge overloaded. • JetStar™ DNA-Binding Cartridge damaged.
JetStar™ DNA-Binding Cartridge Wash	No DNA	DNA	• JetStar™ DNA-Binding Cartridge damaged.
JetStar™ DNA-Binding Cartridge Eluate	DNA	No DNA	• Lysate at improper pH or salt concentration to bind column. • Use correct Elution Buffer (E6) and perform optional elution step.

Continued on next page

Troubleshooting, Continued

Introduction

Review the information below to troubleshoot your experiments with JetStar™ 2.0 Endotoxin-free Plasmid Purification Kits.

Problem	Cause	Solution
Low plasmid DNA yield	Temperature of Lysis Buffer (E2) or Elution Buffer (E6) is too low	Store Lysis Buffer (E2) and Elution Buffer (E6) at room temperature.
	Low copy number plasmid used	Increase the volume of culture processed.
	Lysate at improper pH or salt concentration for DNA binding	Carefully remove all culture medium before resuspending cells. Make sure that the correct volume of Precipitation Buffer (E3) is added.
	JetStar™ DNA-Binding Cartridge damaged	Make sure not to damage the cartridge during the procedure by screwing the cartridge too tight or dropping it.
	No plasmid precipitation (plasmid DNA present in eluate aliquot but none or little plasmid DNA recovered after precipitation)	Make sure to measure correctly the volume of eluate in each centrifugation tube and add exactly 0.7 volume of isopropanol. Make sure to centrifuge plasmid DNA at the appropriate speed and temperature in a swinging rotor.
	Plasmid DNA pellet over-dried	Do not dry the DNA pellet with a vacuum system.
JetFilter clogged	JetFilter clogged by lysate	Let the lysate stand in the cartridge to allow the precipitate to float and form a layer on top of the lysate.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert tubes to mix after adding Lysis Buffer (E2) and Precipitation Buffer (E3). Do not vortex as it can shear genomic DNA.
Additional plasmid forms present	Plasmid DNA permanently denatured	Incubate lysate at room temperature for no longer than 5 minutes after adding Lysis Buffer (E2).
RNA contamination	Lysate at improper pH, salt concentration, or temperature	Carefully remove all culture medium before resuspending cells. Make sure to add the correct volume of Precipitation Buffer (E3) to the lysate.
	Lysate left on JetStar™ DNA-Binding Cartridge too long	Once the lysate is loaded onto the cartridge, avoid delays in processing.
	Lysate droplets remained on walls of cartridge	Wash droplets of lysate from walls of the cartridge with Wash Buffer (E5).
	RNase A digestion incomplete	Make sure RNase A is added to Cell Resuspending Buffer (E1) and Buffer E1 with RNase A has been properly stored at 4°C. Use the recommended volume of Cell Resuspending Buffer (E1).

Accessory Products

JetRack

The JetRack (Cat. no. 210 001) is designed for JetStar™ Maxi gravity flow columns. It can be used simultaneously for 4 Maxi columns. A waste container is included.

For more information, visit www.genomed-dna.com or contact Technical Support (page 35).

Additional Products

The table below lists additional products available from Invitrogen that may be used with kits.

For more information about these products, visit www.invitrogen.com or contact Technical Support (page 35).

Product	Amount	Cat. no.
Luria Broth Base (Miller's LB Broth Base)®, powder	500 g	12795-027
	2.5 kg	12795-084
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Carbenicillin, Disodium Salt	5 g	10177-012
PureLink™ Vacuum Regulator	1 each	K2110-02
PureLink™ HiPure Precipitator Module	10 preps	K2100-21
	25 preps	K2100-22
Quant-iT™ DNA Assay Kit, High Sensitivity	1,000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1,000 assays	Q33130
Qubit® Fluorometer	1 each	Q32857

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless, pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats for your convenience.

DNA ladders are available from Invitrogen for sizing DNA. For more details on these products, visit www.invitrogen.com.

Technical Support

World Wide Web

Visit the website at **www.genomed-dna.com** for:

- Technical resources, including manuals, MSDSs, FAQs
- Complete technical support contact information
- Access to the Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on **www.genomed-dna.com**.

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Purchaser Notification, Continued

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